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# Preliminarly Survey Results and Phylogenetic Analysis for *Tomato Yellow Leaf Curl Virus* and *Potato Leaf Roll Virus* on Tomato Grown in Adana

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**Abstract**

Surveys were conducted at tomato production sites in Adana province in 2019. 53 tomato plants have been collected showing the virus symptom due to determining its prevalence. The collected samples were tested for 13 different viral agents harmful to tomatoes using ELISA and RT-PCR methods. 24.52% TYLCV and 9.43% PLRV were detected from the tested samples. When DNA sequencing comparisons are made from RT-PCR products, 98,74% of PLRV isolates in tomato samples collected from Adana province are homogical similarity with Belgium potato isolate (KX364206.1). Also it clustered at 99.37% similar to same branch with New Zealand (GU002341.1) (BLAST at NCBI).

**Keywords:** Tomato, TYLCV, PLRV, ELISA, PCR

## Introduction

Tomato (*Solanum lycopersicum*) is consumed both as fresh fruit in its own right, as ingredients in many salad recipes or in the form of various foods processed in crushing, whole peeled tomato canning, sliced product, various juices and soups. Tomatoes are consumed in salads, as fresh fruit in their own right, as ingredients in many recipes, or in the form of various processed products. putty, whole peeled tomatoes, chopped produce and various juices and soups. Tomato is a valuable nutrients in many parts of the world and is an economically important agricultural plant.

Tomato is a major plant product and has gained popularity over the last century. It is also grown in almost every country in the world (according to Linnaeus' binomial systematic classification, there are three basic tomato species in them [*L. esculentum, L. peruvianum, L. pimpinellifolium*] and potato (*L. tuberosum*.) is listed under the genus lycopersicon, which also includes. The motherland of tomato is central and South America and its use as a cultured plant started off the coast of Peru (Günay, 1992). Tomato, which was later brought to Anatolia, became a vegetable that was widely grown and consumed (Yazgan and Fidan, 1996). Tomatoes in the Solanaceae family (Solanum lycopersicum) have ranked one of the most important and widely produced crops with a global production increasing by about 45 million tons between 2007 and 2017 (FAO 2017). As of 2017, China is ranked first with 59.6 million tons of world tomato production, India is second with 20.7 million tons of production, Turkey is third with 12.75 million tons (7%) and the United States is fourth with 12.6 million tons of production. 12.75 million tons of tomatoes are produced in Turkey, which includes 8.4 million tons of tableware and 3.7 million tons of tomato paste. In 2018, Antalya is ranked first with 2.410 thousand tons of tomato production, Bursa is ranked second with 1.575 thousand tons and Manisa is ranked third with 975 thousand tons. The highest yield in Turkey is harvested in the Mediterranean Region due to its climate advantage and its greenhouse location. Turkey's tomato production increased by 7% in 2018 compared to 2012 and reached 12.2 million tons. According to TurkStat plant production statistics, tomato production in 2019 increased by 5.7% compared to the former year and amounted to 12.8 million tons.

Despite intensive control efforts to prevent pathogen infec-

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tions in tomatoes, viral diseases are quite difficult to control. Management strategies are becoming increasingly difficult with the constant emergence of new strain or species. Viruses have great potential to adapt to the pressure of natural selection due to their large populations, lack of repair mechanisms facilitating genetic variation in their genomes and their ability to reproduce in a short time (Hanssen et al. 2010). High mutation and recombination ability in viral genomes increases the production of new variants that spread rapidly in the population (Moya et al. 2004). The food content, fruit quality and yield of tomato plants affected by Viral diseases decrease and the shelf life is shortened (Hanson et al. 2016).

In general, virus diseases causes dwarfing, necrosis, deformity, different types of leaf spots (mosaic, ring, speckle, like opening a vein), with a more pronounced effect of the plants by chlorosis and leaf deformation and causes symptoms such as sudden death. These viruses, can be transported vegetatively by production material, pollen and vector insects (Aphids, Whiteflies, Thrips, Mites, Nematodes), mechanically used tools and equipment. There is no effective chemical management control against virus diseases. The absence of a method of combating these diseases in practice increases the importance of virus diseases. Tomato plants grown under cover or under field conditions are particularly exposed to high levels of infections due to the mechanical and seed-borne virus genuses. Tobacco Mosaic Virus (TMV) (Mayer et al. 2009), Pepper Mild Mottle Virus (PMMoV), Tobacco Mild Green Mosaic Virus (MGMT), Tomato Mosaic Virus (ToMV), Tomato Brown Rugose Fruit Virus (ToBRFV) (Fidan, 2020), Tomato Ring Spot Virus (ToRSV), Cucumber Mosaic Virus (CMV), Tomato Spotted Wilt Virus (TSWV), Potato virus Y (PVY), Potato Leaf Roll Virus (PLRV) and Tomato Yellow Leaf Curl Virus (TYLCV), can infect on family Solanaceae.

Tomato Yellow Leaf Curl Virus (TYLCV) is an individual from the genus Begomovirus, in the family Geminiviridae, causing crop losses of over 100% in tomato varieties (Czosnek and Laterrot, 1997). The spread of TYLCV is primarily caused by infected plant material movement or by whiteflies that have TYLCV (Torre et al., 2018). The virus was first spoted in Jordan and Israel in 1939 (Avidov, 1944). It has a wide range of hosts, with several plant families including Solanaceae. TYLCV causes leaf speckling, yellow leaf edges, flower casting and small leaf. In the early stages, infected plants may not bear fruit due to seasonal conditions and variety sensitivity. Its genome formed of circular, single-stranded DNA (ssDNA) about 2.7 kb long (Gronenborn, 2007).

Likewise TYLCV, PLRV is one of the most important viruses that infects members of the Solanaceae family. Among them, the potato culture is the most important host of PLRV. PLRV located within the genus polerovirus in Luteoviridae. Many kind of symptoms depending on the viral strains, host tolerance, duration of infection and also environmental factors. Its symptoms are redness or yellowing in stunted and infected potato plants, and rolling leaves. As well It reduces the number and size of potato tubers with annual world productivity losses of over 20 million tonnes. PLRV formed of single-stranded RNA (+) ssRNA in the (+) sense of the 5.9 kb genome. Potato leaf roll virus can be transmitted experimentally by grafting, aphids in a circulative and non-propagative manner. In aphids, *Myzus persicae* is the most potent vector. Disease management approaches include thermotherapy, tissue culture, resistance breeding, effective vector control and seed potato certification programs. The symptoms of the virus seen in the fields where the crop is grown also show similarities with the symptoms of unblanced fertilization and lack of irrigation in the same cultivated plant (Banttari, 1965).

In field surveys, symptoms caused by viruses and virus-like disease factors were observed in tomato fields. In this study, it is aimed to definitively identify this factor that causes leaf curl symptoms in tomato plants in our region with serological and molecular methods. It was also performed to determine the role of the tomato plant on host virus relationships and to determine the possible risks that PLRV carries in vegetable production.

**Materials and Methods**

**Material**

The plant materials were collected from the fields (Çukurova-14; Yumurtalık-12 and Karataş-27) in Adana province in the fall of 2019 (Figure 1.). 53 samples showing symptoms of dwarfing, redness or yellowing and rolling leaves were tested with serological and molecular techniques at the Virology Laboratory of the Dept. of Plant Protection of the Faculty of Agriculture of Akdeniz University.

**Method**

In the study, Double Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (DAS-ELISA) and Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique were used Due to the fact that when the tomato samples are examined, the symptoms of viral infection come to the fore. For this reason, serological and molecular assays have been designed to provide more precise results instead of general methods such as dsRNA analysis or electron microscopy.

DAS-ELISA

The study was conducted according to Clark and Adams, (1977). Tomato samples were extracted in phosphate buffer solution (PBS) 1/5 (0.2 g $NaN_3$, 0.2 g $KH_2PO_4$, 8.0 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4.12H_2O$, 20 g polyvinylpyrrolidone-25 per L, pH 7.4). ELISA plates were primarily coated with diluted virus-specific antiserum in coatingbuffer (2.93 g $NaHCO_3$, 1.59 g $Na_2CO_3$, 0.2 g $NaN_3$ per L, pH 9.6) and incubated for two hours at 37°C. Following incubation, Washing (PBST) buffer (0.2 g $KH_2PO_4$, 2.9 g $Na_2HPO_4.12H_2O$, 8.0 g NaCl, 0.2 g KCl, 0.2 g $NaN_3$, 0.5 mL Tween - 20 per L) was added to washed plates from crushed samples and overnight at 4°C. Conjugated (IgG) antibody with alkaline phosphatase specific to the diluted virus in a conjugate buffer (2% polyvniylpyrrolidone-25+ PBST + 0.2% BSA (bovine serum albumin, pH 7.4) was added to the re-washed plate wells after overnight. This process was followed by 2 hours of incubation at 37°C. After repeated washing after incubation, substrate buffer containing P-nitrophenylphosphate in (97 ml diethanolamine, 0.2 g NaN3 L-1, pH 9.8) was added to plate wells and kept for 2 hours in dark conditions at room temperature.

The expected absorption values of the test result were measured at 405 nm in ELISA reader. After twice washing

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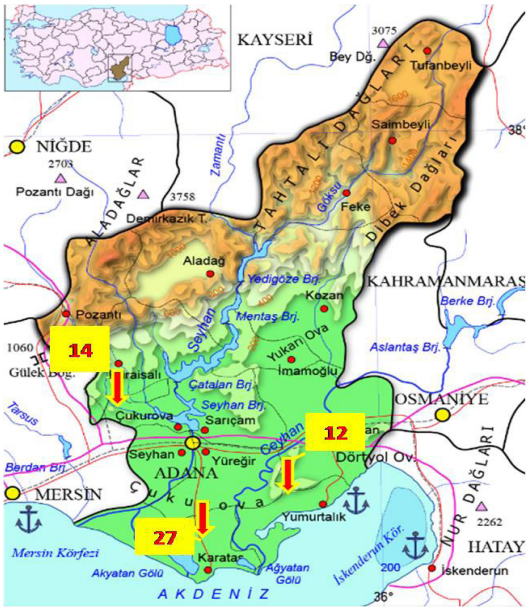


Figure 1 Sample locations and sample numbers of plant materials in Adana (Çukurova-14; Yumurtalık-12 and Karataş-27) http://cografyaharita.com/turkiye_fiziki_harirtalari.html

of plates, the absorbance value of buffer and negative control were considered to be infected with CMV. To diagnose the cause of the disease, leaf samples were assayed with with specific polyclonal antiserum which obtained from BIOREBA AG and Loewe Agdia companies by DAS-ELISA technique against the Cucumber Mosaic Virus (CMV), Pepper Mild Mottle Virus (PMMoV), Tomato mosaic virus (ToMV), Tobacco mosaic virus (TMV), Tomato Ring Spot Virus (ToRSV), Tobacco Mild Green Mosaic Virus (MGMT), Tomato Spotted Wilt Virüs (TSWV), Potato virus Y (PVY), Potato virus X (PVX), and Potato Leaf Roll Virus (PLRV) also known as the common viruses for solanaceae plants.

RT-PCR Method

RT-PCR method was also used in total RNAs extracted according to Astruc et al.,(1996) with total nucleic acid extraction buffer (50mM EDTA pH. 7.0, 500 NaCI, 100 mM Tris-HCI pH.8.0, 10 mM 2. mercapto-ethanol (1/1000) ) saturated at 1:2 (w/v). The positive found samples for PLRV in DAS-ELISA were confirmed by the use of total RNAs extracted from 100 mg leaves in RT-PCR. The same samples were tested with PCR against TYLCV due to suspicion of symptom type.

The spesific primer sets TYv2664 (5`-ATTGACCAAGATTTTTACACTTATCCC-3') and TYc138 (5'-AAGTGGGTCCCACATATTGCAAGAC-3`) were used to clone a 316 bp sub-genomic region to identify TYLCV infection in this study (Anfoka et al. 2005) (Table 1). For the same reason of PLRV, cDNA amplicons have been cloned with primer sets Forward 5'-CGCGCTACAGAGTTCAGCC-3`) and Reverse 5` - GCAATGGGGGTCCAACTCAT-3`) reported by Singh et al. (1995).

The PCR and RT-PCR studies were conducted separately. The PCR studies were conducted using the Thermo Sci. Verso 1-Step RT-PCR *ReddyMix Kit*. In total volume of 50 µL; 2X 1-Step PCR ReddyMix 25 µL, 1X RT Enhancer 2.5 µL, Verso enzyme Mix 1 µL, , Reverse primer (10 µM) 1 µL 200 nM, Forward primer (10 µM) 1 µL 200 nM, sample RNA 1-5 µL 1 ng, nuclease free water content to be completed according to total volume of 50 µL has been adjusted as suggested by the company.

One-Step RT-PCR stage for PLRV (50°C 15 min. cDNA synthesis single cycle, 95°C 2 min., verso inactivation single cycle; 95°C, 30 sec. denaturation; 52°C, 30 sec. annealing; and 72°C, 45 sec. extension 35 cycles) and final extension was performed in a single cycle of 10 minutes at 72 ° C (Singh et al. 1995). For TYLCV inactivation single cycle at 94°C for 5 min; 30 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min.; for the termination of reaction, one cycle at 72°C for 10 min. performed in the form (Anfoka et al. 2005). cDNA bands belonging to the causal agents were observed by staining solution (ethidium bromide) after electrophoresis in agarose gel (1.5%).

**Results and Discussions**

TYLCV and PLRV symptoms (curling, systemic chlorosis, leaf deformation, lamina narrowing) were determined in the leaves of the tomato plants sampled during the study (Figure 2). Symptoms such as yellowing, stunting and leaf curling on TYLCV infected tomato plants along with important production losses were shown in tomato cultivation (Kil et al., 2016; Papayiannis et al., 2010). Other than tomato, some cultivated plants including Eustoma, Common bean Pepper and Cucurbits have been underlined as TYLCV hosts (Anfoka et al., 2009; Kil et al., 2016). Polston et al.(1994) similarly reported that symptoms (reduced leaf size, yellowing and distortion and cupped inward) on surveyed tomato plants were shown. Severe stunting appears with time. TYLCV is a limiting factor for cultivation (Lapidot and Friedmann, 2002). The infection time can increased yield losses (aproximataly 100%) (Lapidot and Polston, 2006). Dalmon anf Marchoux, 2000 declared that TYLCV has 11 natural and 30 experimental host species. They also underlined susceptible hosts of TYLCV as tomato, zinnia, bean, paprika, tobacco, lisianthus species. In addition to cultivated species, some wild or native species (*Solanum*

*nigrum, Datura stramonium, and Malva sp*.) which are common in France, are stand as natural infection reservoir (with or without symptoms).

In Turkey, Yilmaz (1978) studied disease and TYLCV symptoms . Karut et al.2012 have detailed study on vector characterization on different host plant in Adana. Fidan et al.2019 collected some symptomatic samples from tomato plants (with yellowing, stunting, upward leaf curling, distortion and symptoms). the same researchers brought clarity to the connection between tylcv vectors and the transport of virus races. they also emphasized that vector-transportable diseases such as TYLCV can make the transition to non-host species to this day.


Figure 2. In tomato plants found to be infected with TYLCV and PLRV in the survey (A.- Tylcv-induced chlorosis, yellow leaf curl and dwarfing; B., C., D.- PLRV-induced leaf curl and distortion) symptoms

On the potato leaves, yellowing and rolling symptoms of PLRV are common, had been reported by different researchers (less commons are papery and leathery) (Harrison, 1984; Radcliffe and Ragsdale, 2002). In Pakistan for PLRV, the yield losses were dedicated as around 90% (Bhutta and Bhatti, 2002). Batool et al 2011 reported some PLRV symptoms as dwarfing plants with yellowing and rolled leaf inward. PLRV detected on different alternative cultivated host from solanacea which is Tree tomato (*Solanum betaceum*) were showed yellow mosaic and curling inward of the leaves on stunted growth. After the first infection, symptoms of PLRV are recogniseble in leaves with upward rolling, slightly pale and may show purpling arrived to stunting Anoymous, 2018.

In Turkey, Güner ve Yorgancı (2006), detected PLRV from sympytomatic and asymptomatic samples of potato by ELISA and biological indexing in Niğde and Nevşehir provinces. They had been recorded both single infection of PLRV and mixed infections with other potato viruses (PVY, PVX, PVS, PVA). Some critical symptoms as mottling, vein clearing and banding. As well distortion, local lesion were underlined in same study. In an other study, Yardımcı et al. (2015) detected the PLRV on potato plants with common potato viruses as mixed infection by ELISA.

DAS ELISA;

As a result of serology studies, chlorosis, lamina narrowing, leaf deformation and curling in the leaves, symptoms was found to be related with PLRV.

There was no positive response to any other viral agent serologically with other antiserums used during the study. Positive samples were also tested against TYLCV and PLRV by PCR method. Thus, both confirmation of PLRV results and mixed infection cases with TYLCV were examined. However, no mixed infection was detected. In the study, PLRV disease incidence was determined as 9.4% and TYLCV 24.52% in the collected samples (Table 1). In addition to the detection of TYLCV, the detection of PLRV in predominantly potato species rather than tomato, has point out vector transportation. The molecular origin of this factor, which may have important outcomes in terms of virus ecology and epidemiology, was a priority in the study. Similarly, Gugerli (1979) stated that PLRV and PVA with the ELISA method could easily be used in routine testing in potatoes and in test plants. Jafarpour et al. (1988) stated that in their study with the ELISA test they detected 3-4% PVS, PVX and 9% PLRV in certified seeds and commercial potato tubers produced in Australia.

Ali et al.(2002), in a study conducted in different ecoregions of Pakistan, they found that PLRV and PVY viruses, which cause significant problems in potatoes grown, were detected 0-71.0% in autumn respectively. Whitworth et al. (1993) attempted to detect PLRV using observational examination, direct tissue bloting and ELISA tests. In Turkey, Dolar et al., (1976), found PVX, PVY, PLRV, Aucuba Baciliform Badnavirus (AuBV) and witch broom virus and their transmission rates in potato r regions of Adana, Içel, Gaziantep, Hatay, Antalya and Kahramanmaraş provinces. It has been reported by Çıtır (1982) that seed potato tubers in and around Erzurum are infected with PVX, PVY, PLRV, PVS and low percentage PVA. Özbayram ve Yorganci (1989), conducted a survey of PVX, PVS, PVA, PVY and PLRV in 23 centers in Turkey, indicated that 32.08% of PLRV infections were present. Yilmaz et al. (1990), determined that the prevalence of the disease ranged between 0.8% and 17.68% in 1997 and 1987 and that it could be used in tubers in tests as a result of the study conducted by ELISA test for leaf curl virus in early harvest potatoes grown in Çukurova region. Sökmen et al. (2005) in their study, they determined the Potato X Virus (PVX) and Potato Y Virus (PVY) with DAS-ELISA in seed potatoes in the fields. However, none of the potato tuber samples tested were found to be infected with Potato Leaf Curl Virus (PLRV). Al-Ali et al. (2016) reported that 45 out of 50 plants were infected with TYLCV after testing with DAS-ELISA method for symptoms such as upward curling, chlorosis, mosaic, dwarfing in plants

and deformation in fruits and leaves, one of the most important vegetables grown in Kuwait. The existence of TYLCV in our country was determined by Yilmaz (1978). Molecular characterization was first performed by Köklü et al, (2006). Yilmaz (2016), in his study in Hatay province, found 26% of infection with TYLCV in samples collected in organic tomato cultivation under cover.

Table 1. Assay results for collected tomato leaf samples

| VIRUS SPECIES | ASSAY | *RESULT |
|---|---|---|
| TRSV; Tobacco Ringspot Virus | DASELISA | - |
| ToRSV; Tomato Ringspot Virus | DASELISA | - |
| PMMoV; Pepper Mild Mottle Virus | DASELISA | - |
| TMGMV; Tobacco Mild Green Mosaic Virus | DASELISA | - |
| CMV; Cucumber Mosaic Virus | DASELISA | - |
| TMV; Tobacco Mosaic Virus | DASELISA | - |
| ToRSV; Tomato Ring Spot Virus | DASELISA | - |
| ToMV; Tomato Mosaic Virus | DASELISA | - |
| PLRV; Potato Leaf Roll Virus | DASELISA/RT-PCR | ++(5 sample) |
| TSWV; Tomato Spotted Wilt Virus | DASELISA | - |
| PVX; Potato Virus X | DASELISA | - |
| TYLCV; Tomato Yellow Leaf Curl Virus | PCR | +(13 sample) |
| PVY; Potato Virus Y | DASELISA | - |

*(-) Negative: not infected, (+) Positive: infected

Molecular Studies

All 5 samples that received PLRV positive results in the ELISA test were evaluated in the study with RT-PCR and the same positive results were obtained as the ELISA test results. The 336 bp cDNA amplicons of PLRV were verifed with 1.5 agarose jel electrophoresis (Figure 3). All 53 tomato samples collected in the study were PCR tested against TYLCV and 13 of them showed positive results*.

* Since the detailed data of TYLCV positive samples will be a basis for a separate study, the molecular data in particular is not detailed in the scope of the publication.

The presence of PLRV in tomato culture has been determined by any laboratory technique. However, molecular determinations associated with PLRV were carried out on potato isolates (Bostan et al., 2004; Ghazal et al., 2009). Sivaprasad et al., (2016) found positive results on *Solanum betaceum* in their testing with both ELISA and RTPCR. The study is also one of the rare and special evidence that PLRV can infect different Solanaceae hosts other than potatoes.

Fidan et al. (2011) found that TYLCV-IL, TYLCSV (TYLCV-Sardinia) and TYLCV-Mild strains were present in our country using molecular diagnostic methods. Atalay (2018) identified two strains with TYLCV-specific primary pairs (TYLCV-IL, TYLCV-Mld) in a study conducted in 2017 to investigate variants of TYLCV in Adana and Mersin provinces. The same researcher revealed that many viral variants of TYLCV threatening tomato cultivation in the Mediterranean region.

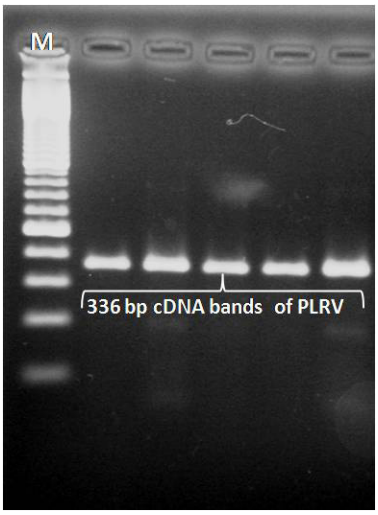


Figure 3. 336 bp cDNA bands of PLRV infected tomato samples by the primer pairs according to Singh et al. (1995); M:;DNA ladder (%1,5 agarose jel)

The evolutionary history was inferred using the Neighbor-Joining procedure. The optimal tree where in the related taxa grouped together in the bootstrap test (1000 repeats) are appeared close to the branches were determined. The evolutionary separations were processed utilizing the p-separation procedure The research has been incorporated 18 nucleotide groupings. All gaps and missings were eliminated with. There were a total of 212 circumstances in the last dataset. Evolutionary assessments were directed in MEGA7.

When the sequences compared, PLRV-Ad isolate, are clus-

tered in Group 1, subgroup 1a with other isolates from NCBI, shown maximum 98,74% similarity with Belgium potato isolate (KX364206.1). Also it clustered in same branch with New Zealand (GU002341.1) 99.37% similarity (Şekil 4.) .

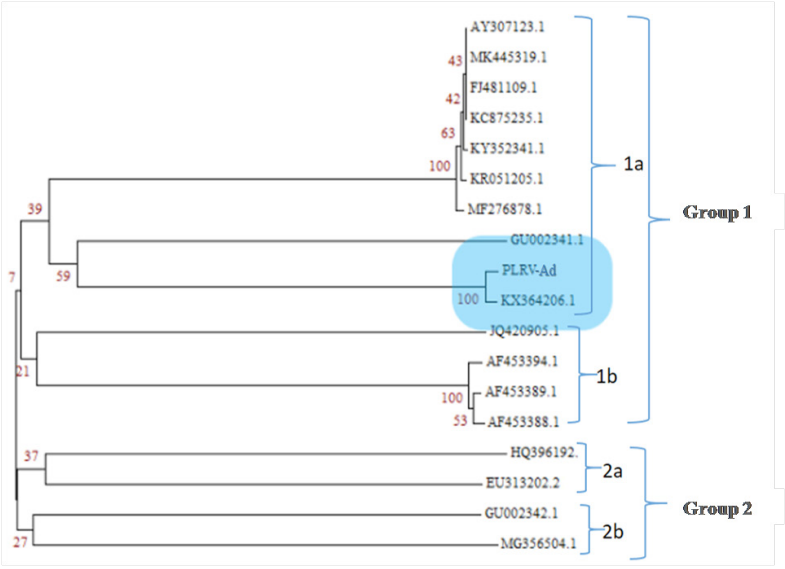


Figure 4. After haplotype examination, phylogenetic trees were built with MEGA 7 program. Related 18 confines were chosen by utilizing

**Conclusion**

The most important protection methods are to avoid disease resistance of virus diseases, to eliminate sources of inoculum and to fight vectors effectively due to the lack of chemical management. All necessary methods of controlling viruses should be used and economic losses should be reduced at both local and regional levels.

Virus free certified basic material should be used. Weeds that host for viruses and aphids at the same time need to be eliminated in seedling field, greenhouse or land. Plants (tomato, pepper, lettuce, tobacco, etc.) that are mainly the main host of PLRV, including the potato culture) should not be grown side by side. This plays a key role for the epidemiology of all plant virus diseases. Effective vector control must be done by considering transitions through vectors to wild host species around the land.

It is concluded that PLRV was determined for the first time with DAS-ELISA and one step RT-PCR in tomato samples collected from Adana province and was evaluated decisively because it is not only a cultured plant with intermediate host potential but also an important cultured plant in economic terms. Although the Tomato Yellow Leaf Curl Virus is one of the most prominent of these, the Potato Leaf Roll Virus (PLRV), which is one of the rare factors in tomato culture, has the potential to be economically and ecologically important.

**Compliance with Ethical Standards**

**Conflict of interest**

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Author contribution**

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

**Ethical approval**

Not applicable.

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**Data availability**

Not applicable.

**Consent for publication**

Not applicable.

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